

AWARD NUMBER: W81XWH-15-1-0084

TITLE: Cancer Risks Associated with Inherited Mutations in Ovarian Cancer
Susceptibility Genes Beyond BRCA1 and BRCA2

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REPORT DATE: May 2016

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE May 2016		2. REPORT TYPE Annual Report		3. DATES COVERED 04/15/2015 - 04/14/2016	
4. TITLE AND SUBTITLE Cancer Risks Associated with Inherited Mutations in Ovarian Cancer Susceptibility Genes Beyond BRCA1 and BRCA2				5a. CONTRACT NUMBER W81XWH-15-1-0084	
				5b. GRANT NUMBER W81XWH-15-1-0084	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Elizabeth Swisher E-Mail: swishere@uw.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Washington 4333 Brooklyn Ave NE Box 359472 Seattle, Wa 98195				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Ovarian, peritoneal and fallopian tube carcinomas (OC) are the most deadly of the gynecological cancers. Our data indicate that at least 20% of unselected OC is hereditary and that 20-25% of inherited mutations occur in genes other than <i>BRCA1</i> and <i>BRCA2</i> . The large fraction of OC associated with inherited mutations in a variety of genes provides an important opportunity to reduce OC mortality. Maximizing the benefit from OC risk assessment and prevention requires an improved understanding of the penetrance of OC genes beyond <i>BRCA1/2</i> . Furthermore, minimal data exist regarding the hereditary component of OC, including <i>BRCA1/2</i> , in non-white populations. The objective of this study is to define the genetic causes of hereditary OC in African Americans (AA) as well as the spectrum of cancers, the age of onset, and the relative risk associated with mutations in non- <i>BRCA1/2</i> genes. In year 1, we have enrolled and sequenced 136 high risk probands and 9AA probands with OC for 45 known or candidate OC genes. We have also exome sequenced one families. We continue to enroll probands and their relatives to better understand the genetic contribution to ovarian cancer.					
15. SUBJECT TERMS ovarian cancer, drug resistance, rucaparib, phase 2, DNA repair, homologous recombination, nonhomologous end-joining (NHEJ), poly(ADP-ribose) polymerase, BRCA1, BRCA2, PARP1					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	9	19b. TELEPHONE NUMBER (include area code)

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1. INTRODUCTION

Ovarian, peritoneal and fallopian tube carcinomas (OC) are the most deadly of the gynecological cancers and can be considered together as one entity. While women with early stage OC have an excellent chance of cure, attempts to improve early detection have been largely ineffective. In contrast to surveillance, surgical prophylaxis with risk-reducing salpingo-oophorectomy (RRSO) reduces OC mortality in high risk women. Inherited mutations in *BRCA1* and *BRCA2* (*BRCA1/2*) account for about 15% of OC. Inherited loss of function mutations in other related genes account for another 5-6% of cases, but less is understood about the OC risk associated with mutations in these genes. Furthermore, there are other OC genes that have not yet been discovered. Our hypothesis is that rare, inherited, damaging mutations in genes other than *BRCA1/2* confer a relatively high cancer risk that would warrant age appropriate surgical prophylaxis. A better understanding of the etiologic contribution from, and penetrance of, genes other than *BRCA1/2* to hereditary OC is needed to guide clinical decision-making and to optimize recommendations for OC prevention. Our overall goal is to refine the understanding of inherited OC susceptibility, emphasizing genetic variation in diverse racial populations and genes other than *BRCA1* and *BRCA2*. We will achieve these objectives through two specific aims:

Aim 1: Identify rare variants in OC susceptibility genes other than *BRCA1/2* in women with ovarian, fallopian tube or peritoneal carcinoma who have an increased likelihood of genetic risk.

Aim 2: Identify the genetic contribution of many genes to OC susceptibility among African American women with ovarian, fallopian tube or peritoneal carcinoma.

2. KEY WORDS

ovarian cancer, *BRCA1*, *BRCA2*, cancer susceptibility, *RAD51C*, *RAD51D*, *PALB2*, *BRIP1*, *BARD1*, African-American, familial, hereditary

3. ACCOMPLISHMENTS

The submission of UW IRB approvals and related material for DOD's HRPO approval was completed in year 1.

The first goal was to enroll and BROCA sequence 100 high risk probands in year 1 and that has been exceeded (N=136) as detailed below. Our BROCA targeted sequencing assay includes 11 known OC genes: *BRCA1*, *BRCA2* (*FANCD1*), *BARD1*, *BRIP1* (*FANCI*), *RAD51C* (*FANCO*), *RAD51D*, *PALB2* (*FANCO*), *MSH2*, *MLH1*, *MSH6*, *PMS2*, 9 other known breast cancer genes: *ATM*, *CHEK2*, *FAM175A* (*abraxas*), *FAMCM*, *NBN*, *PTEN*, *RECQL*, *TP53* and *XRCC2*) and 25 other candidate genes in the Fanconi anemia-BRCA pathway: *ATR*, *BABAM1*, *BAP1*, *BLM*, *BRCC3*, *BRE*, *CHEK1*, *ERCC1*, *ERCC4* (*FANCD2*), *FANCA*, *FANCB*, *FANCC*, *FANCD2*, *FANCE*, *FANCF*, *FANCG* (*XRCCC9*), *FANCI*, *FANCL*, *GEN1*, *MRE11A*, *RAD50*, *RAD51*, *RBBP8* (*CtIP*), *SLX4* (*FANCP*), *UIMC1* (*RAP80*). All damaging mutations have been confirmed with Sanger Sequencing.

All 136 enrolled high risk probands had ovarian, fallopian tube, or primary peritoneal carcinoma confirmed on a pathology report. 81 patients had a first or second degree relative with ovarian carcinoma and 48 had a second invasive non-skin cancer. Seven patients were enrolled with a known mutation in a gene of interest including 1 each with a mutation in *RAD51D*, *RAD51C*, *PALB2*, *BARD1*, one with *PMS2* and *PALB2*, and two with *PPM1D* germline mutations. Of the 81 patients with a close relative with ovarian cancer, sequencing results are pending on 5. Of the remaining 76 patients, damaging mutations were identified in 17 (22.3%) including 6 *BRCA1*, two each in *ATM*, *BRIP1*, and *NBN* and 1 each in *BRCA2*, *PMS2*, *BLM*, *RAD51C* and 1

with mutations in *MSH6* and *RAD51D*. Of the 48 patients with a second cancer, most had an invasive breast cancer, but there was a variety of other cancers including cholangiocarcinoma, colorectal, uterine, and thyroid. For these patients, sequencing results are pending in 4 cases, and damaging mutations were identified in a known or suspected ovarian cancer genes in 7/44 (15.9%) including 3 *BRCA1* and 1 each *BLM*, *BRCA2*, *PALB2*, and *RAD51C*. Notably, many of these patients are referred by genetic counselors after having received negative genetic testing results. Therefore, the mutation rate as expected is lower than would be expected in an untested population.

A second goal was to enroll and exome sequence 12 BROCA negative families in year 1. To date we have only exome sequenced 1 family. We decided to postpone the majority of the exome sequencing until year 2 or 3 in order to select the most informative families of all enrolled. Sequencing data for that family is currently under analysis. One barrier we have faced, is that it is challenging to get tissue on more than one OC patient in a family based on the high mortality of OC.

A third goal was to enroll and sequence 50 African-American (AA) women with OC. Enrollment of AA women has lagged behind our goal. Currently we have enrolled 13 AA subjects, and completed sequencing on 9. Of the 9 AA OC patients sequenced to date, 8 patients had no damaging germline mutations in any genes. One patient had a frameshift mutation in *TP53*. That patient had a history of both colon and ovarian cancer

Opportunities for training and professional development has the project provided?

Nothing to report

Dissemination of Results

Nothing to report

Plans during the next reporting period.

We will continue to recruit subjects and family members for both aims. We will use BROCA for targeted sequencing and select the most informative families for exome sequencing. For dead family members, we will obtain tissue from pathological archives when available with approval from next of kin to increase the number of ovarian cancer patients available in families for sequencing (particularly for exome sequencing).

4. IMPACT

Impact on the principal discipline

Nothing to report

Impact on other disciplines

Nothing to report

Impact on technology transfer

Nothing to report

Impact on society

Nothing to report

5. CHANGES/PROBLEMS

Changes in approach

Nothing to report

Problems or delays and plans to resolve them:

Despite having a number of collaborative centers who agreed to refer African American patients, AA patient accrual is lagging. A major reason for this has been the increased use of universal testing for all OC patients including AA women. Centers are more motivated to refer patients who cannot otherwise get genetic testing financially covered. We have been actively encouraging our collaborators to refer AA OC patients, even those who have undergone clinical testing. We are considering submitting a modification to the IRB to recruit AA more broadly through social media and/or AA-oriented magazines (depending on cost). We are also reaching out to advocacy groups (Bright Pink, OCRFA, NOCC, FORCE) to get the word out about the study. Lastly, we plan to reach out to additional cancer centers, particularly in regions that have a higher fraction of AA patients with OC and ask them to partner with us on this important aim. As a backup strategy in case of inadequate enrollment, we may request anonymized DNA from AA OC patients who participated on clinical trials with the Gynecologic Oncology Group.

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

6. PRODUCTS

Publications, conference papers, and presentations

Nothing to report

Website(s) or other Internet site(s)

Nothing to report

Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

Nothing to report

Other Products

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- What individuals have worked on the project?

Name:	Elizabeth Swisher MD
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0003-2331-0434
Nearest person month worked:	1
Contribution to Project:	Dr. Swisher is directing all aspects of the project including IRB oversight, recruitment, sequencing analyses, and data interpretation
Name:	Maria Harrell, PhD
Project Role:	Staff scientist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Dr. Harrell is overseeing all sequencing including quality control.
Funding Support:	
Name:	Ming Lee PhD
Project Role:	Bioinformaticist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Dr. Lee runs the bioinformatics pipeline for the next generation sequencing.
Name:	Kathy Agnew
Project Role:	Staff scientist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1

Contribution to Project:	Ms. Agnew manages all incoming samples, keeps the study database, communicates with referring provers and generates result letters.
Funding Support:	
Name:	Marc Radke
Project Role:	Staff scientist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Mr. Radke preps all sample, extracts DNA and creates library pretps for DNA sequencing. He performs Sanger sequencing validations.
Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Yes. Dr. Swisher's support on the following grants has ended:

Ovarian Cancer Research Foundation (Swisher) 1/1/2013-12/31/2015 3% FTE
The FA-BRCA pathway and Response to PARP Inhibitors and Platinum in Ovarian, Tubal, and Peritoneal Carcinomas
Program Project Development Award
Role: Principal Investigator
The objective is use using existing clinical samples and xenografts to develop a biomarker of cancer response to PARP inhibitor therapy in collaboration with two co-investigators at Mayo Clinic.
Contact: Sarah DeFeo, Director of Scientific Affairs
Ovarian Cancer Research Fund
14 Penn Plaza, Suite 1710
New York, NY 10122

And Dr. Swisher has the additional new support as follows:

American Association of Cancer/Stand Up To Cancer (D'Andrea, Swisher)
Ovarian Cancer Dream Team 6/1/15-5/31/19 2.4 calendar months
DNA Repair Therapies for Ovarian Cancer
Role: Co-leader

The goal of this proposal is to exploit defects in DNA repair in ovarian cancer to develop novel therapies and prevention strategies.

Specific Aims:

Specific Aim 1: Characterize mechanisms of sensitivity and resistance to PARP inhibitors that can identify individual OCs that are hypersensitive to PARP inhibitor monotherapy

Specific Aim 2: Evaluate novel drug combinations that extend the use of PARPi to HR-proficient OC.

Specific Aim 3: To develop OC genetic testing and surgical prevention trials that could serve as a model for future large scale genetic testing and OC prevention.

Contact:

Fei Duan, PhD

program administrator, Scientific Review and Grants Administration

American Association for Cancer Research

615 Chestnut Street, 17th Floor

Philadelphia, PA 19106-4404

NIH NCI R01 CA190423 (Kaufmann) 3/1/15-2/28/20 1.2 calendar months

Mechanisms of PARP Inhibitor Resistance in Ovarian Cancer

Role: Co-investigator

The goal of this study is to identify various molecular alterations that explain PARP inhibitor resistance in patients with cancer.

Specific Aims:

Aim 4 (on which D. Swisher is participating): Examine *BRCA1/2* reversion mutations, genomic scarring, HR and NHEJ pathway alterations as predictive biomarkers in a unique set of pretreatment biopsies from pts with *BRCA1/2*-mutant breast cancers receiving single-agent PARPi therapy.

Contact:

Karen Theis

Mayo Clinic

200 First Street SW; Plummer Building 6

Rochester, MN 55905

Note: There is no overlap between Dr. Swisher's efforts on the above projects or with the current project.

What other organizations were involved as partners?

Nothing to report

8. APPENDICES

None